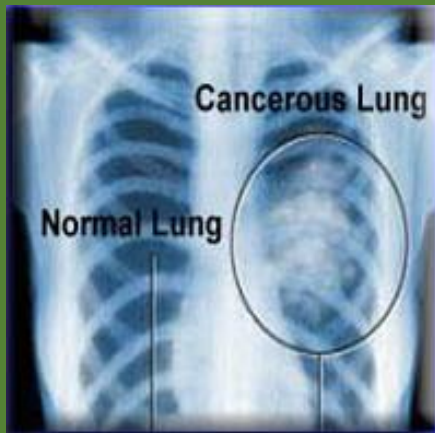
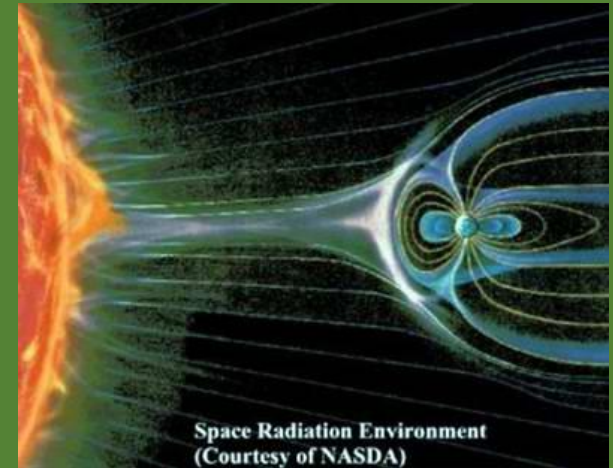


# Transcriptomics, DNA Damage and DNA Damage Response in Space

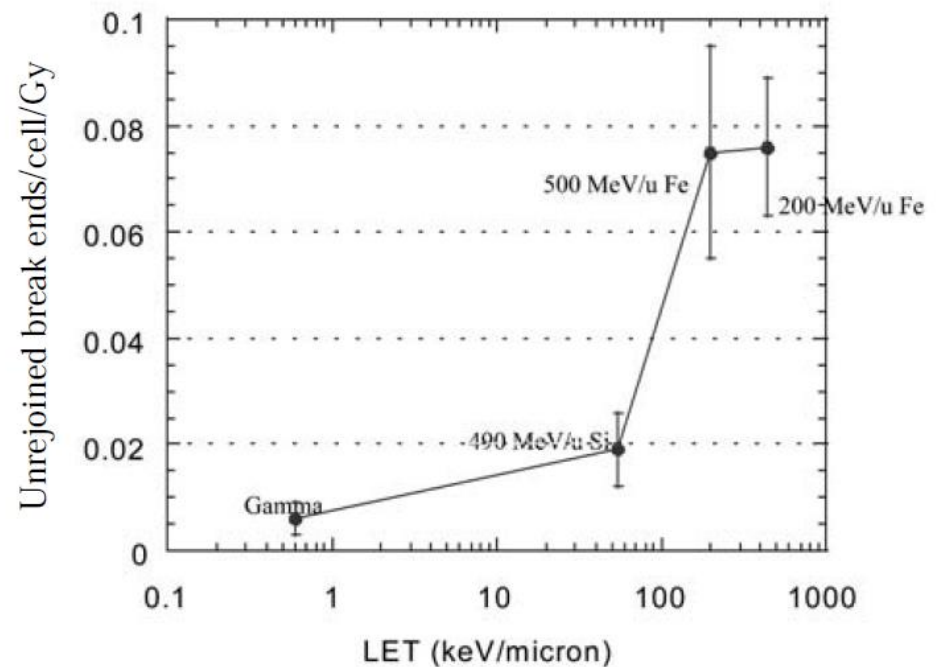
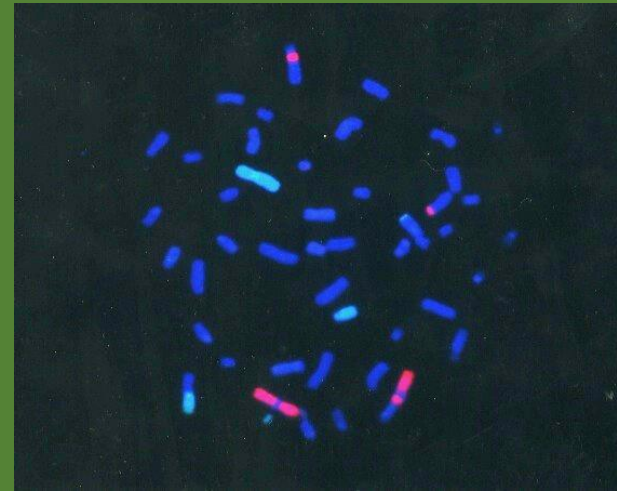
Honglu Wu  
NASA Johnson Space Center  
Houston, Texas, USA

# Space Radiation Risks

- ***Carcinogenesis (morbidity and mortality risk)***
- ***Acute and Late Central Nervous System (CNS) risks***
  - ✓ immediate or late functional changes
- ***Chronic & Degenerative Tissue Risks***
  - ✓ cataracts, heart-disease, etc.
- ***Acute Radiation Risks*** – sickness or death



# NASA Space Radiation Laboratory at BNL



Unrejoined chromosome breaks after low- and high-LET radiation exposure in human fibroblast cells. Wu et al. 2002

# Combined effects of radiation and spaceflight factors

## Experimental design

- Exposure pre-flight
- Exposure during flight
- Exposure post-flight

## Effects

- Synergistic - enhanced
- Additive - same
- Antagonistic - reduced

Chromosome aberration frequencies in pre- and post-flight astronaut lymphocytes irradiated in vitro with low-LET radiation  
(Wu et al. Phys. Med. 2001)

Nission: STS-103

Duration: 8 days

Blood draw schedule:

10 days before launch, JSC, kept at 4 C for 1 day before exposure

0 days after landing, KSC, kept at 4 C and received next day. Kept at 4 C before exposure

14 days after landing, KSC, kept at 4 C for 1 day before exposure

Irradiation: Whole blood was irradiated to gamma rays

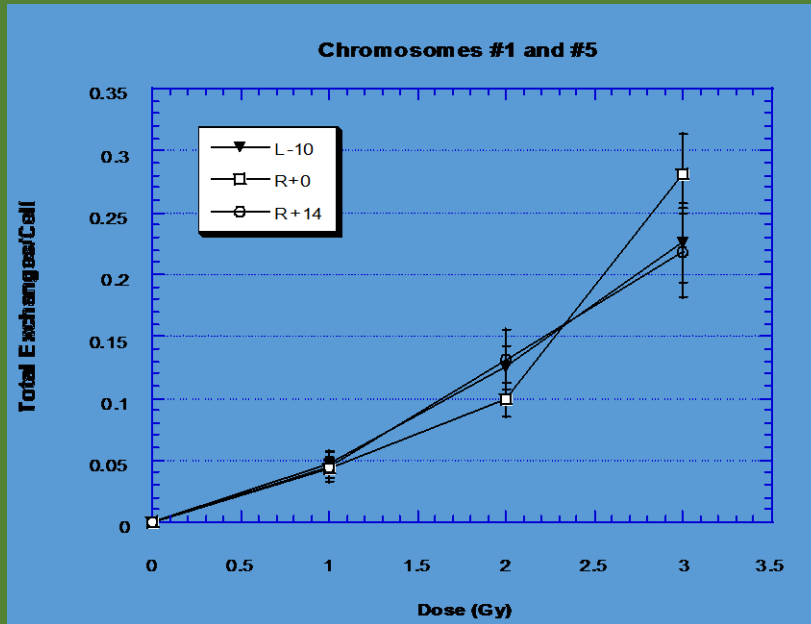
Procedure: Whole blood was stimulated to grow with PHA in growth medium and chromosomes were collected following standard procedures.

Chromosome analysis: Chromosomes #1 and #5 were painted.

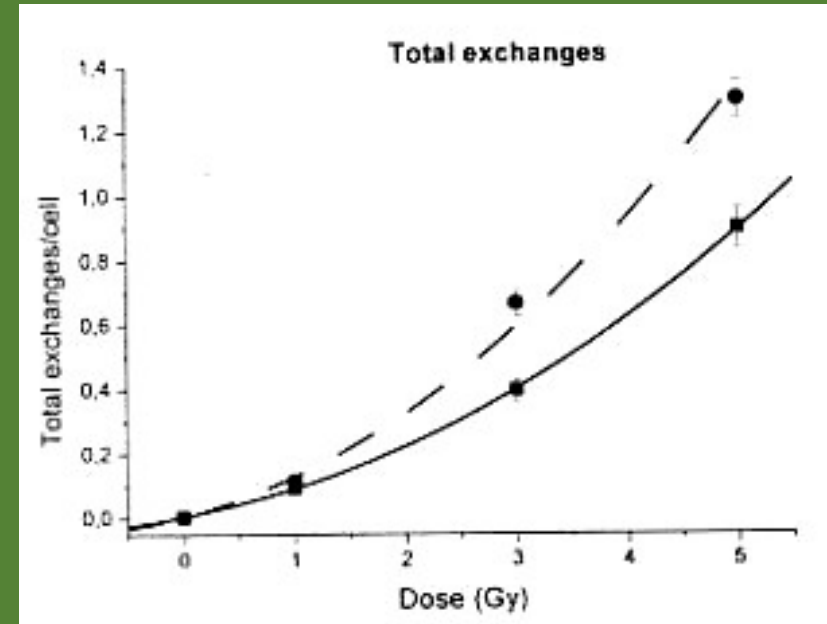




Wu et al. 2001

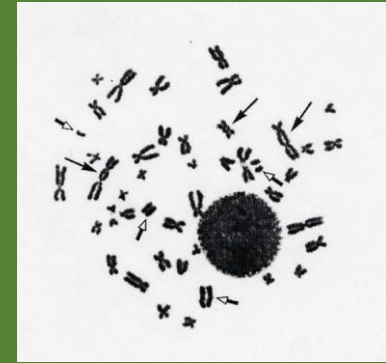


Greco et al. Adv. Space Res. 2003

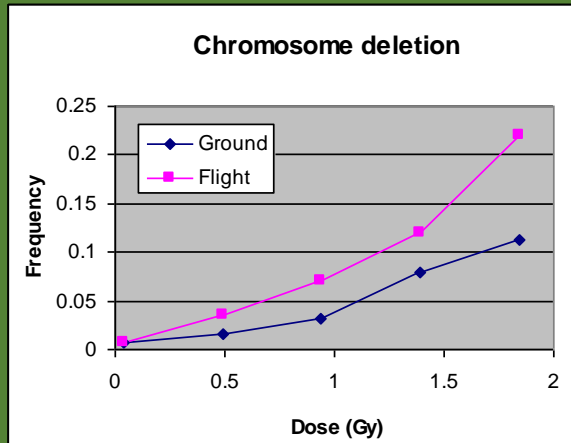


- Post-flight blood was collected 3 days after landing
- Samples were exposed to X-rays

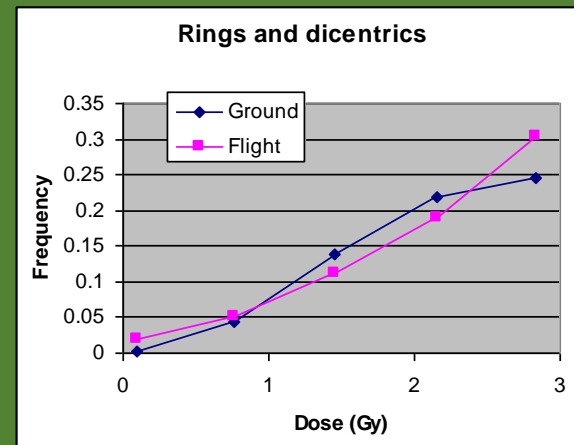
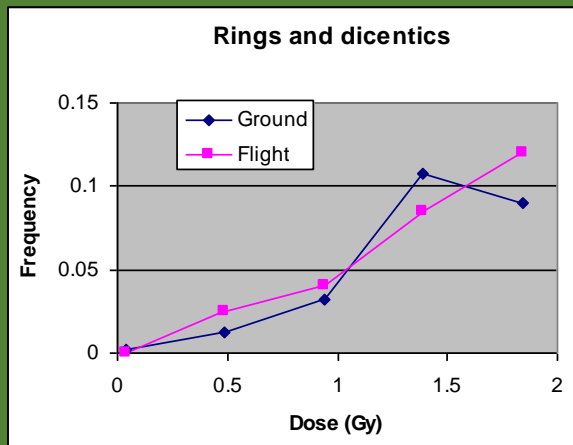
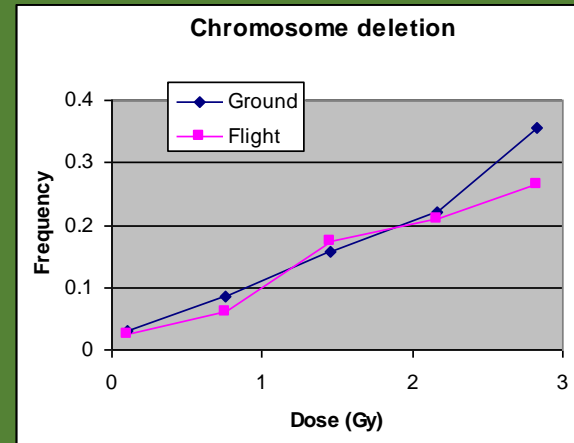
# Chromosome aberrations in lymphocytes induced by beta particle exposure in flight (Bender et al. Rad. Res. 1967, 1968)



## Gemini-3



## Gemini-11



Mission Duration  
Temperature

Ambient

4 hr 52 min

4 day 1 hr 56 min

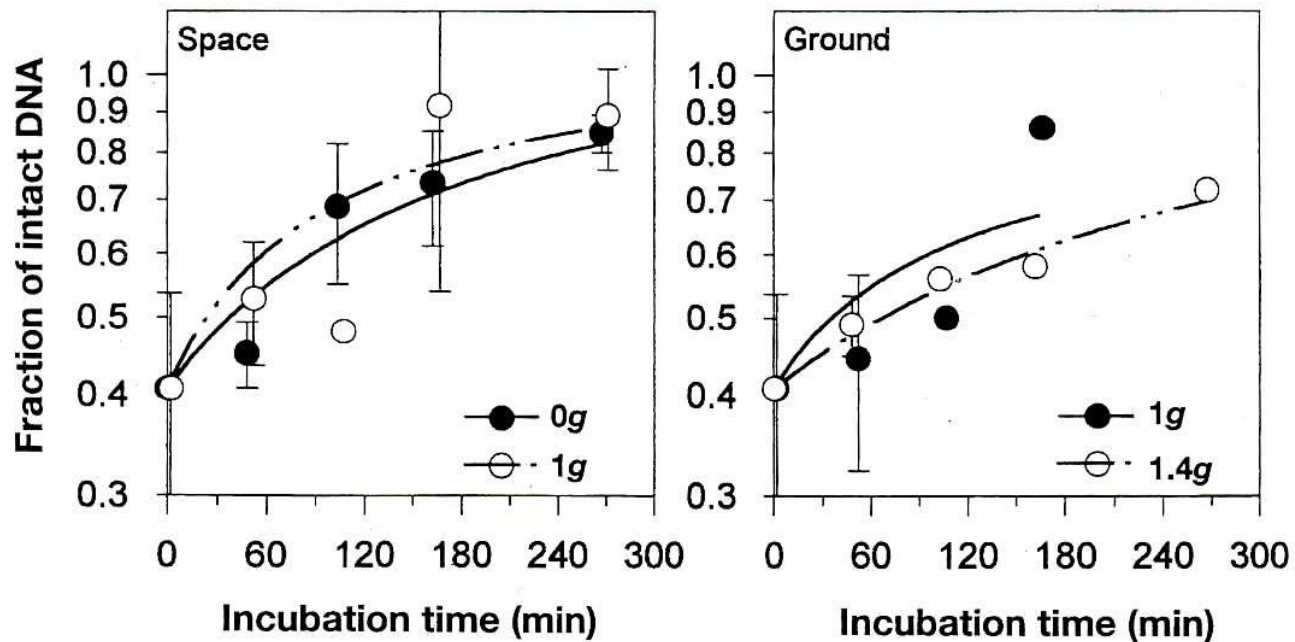
Refrigerated-ambient

Cell: Human fibroblasts

Exposure: X rays. Ground – pre-flight

End point: DNA repair in flight

Source: Honeck et al. Rad. Res. 1997



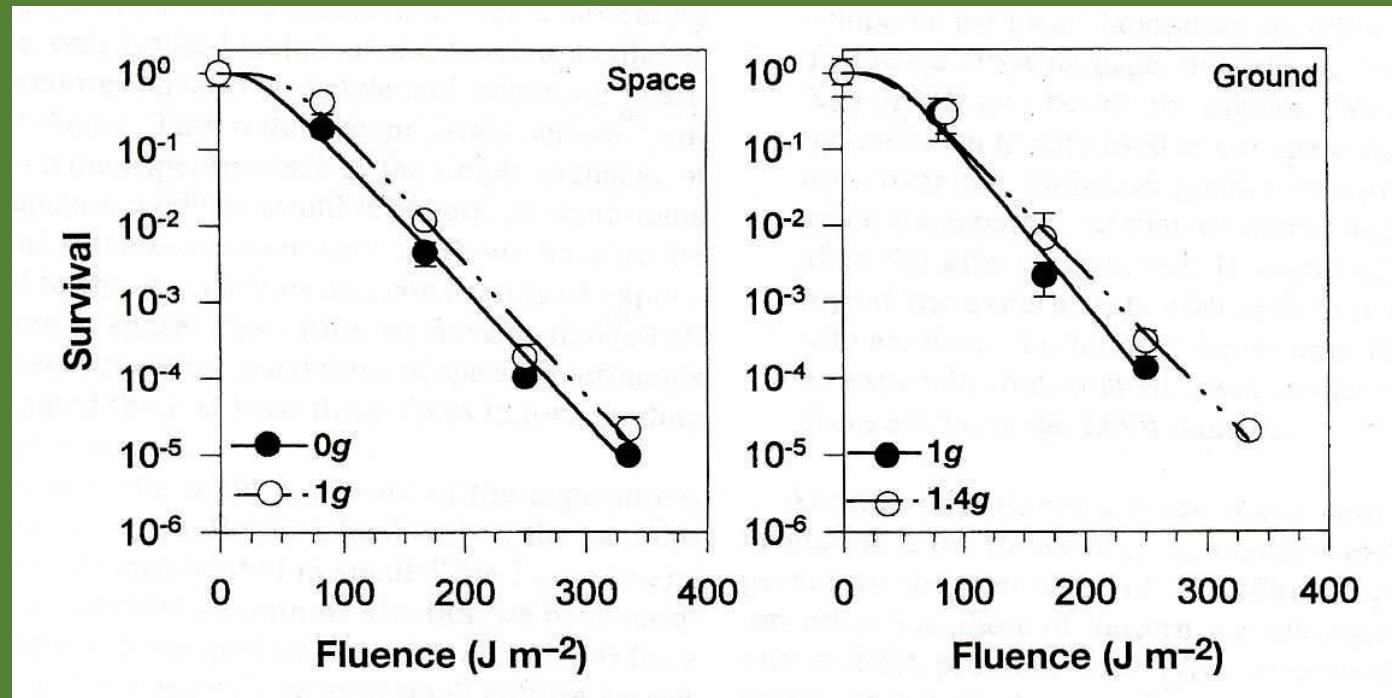


Cell: *B. subtilis* (Bacteria)

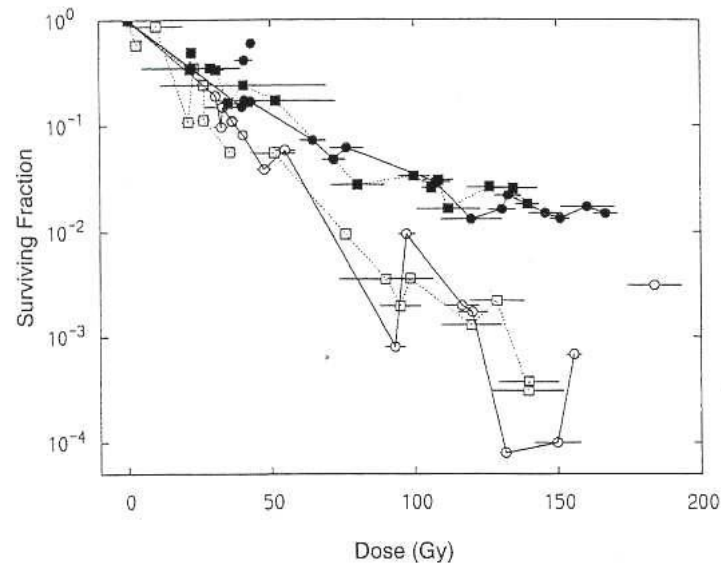
Exposure: UV. Ground – pre-flight

End point: Survival

Source: Honeck et al. Rad. Res. 1997

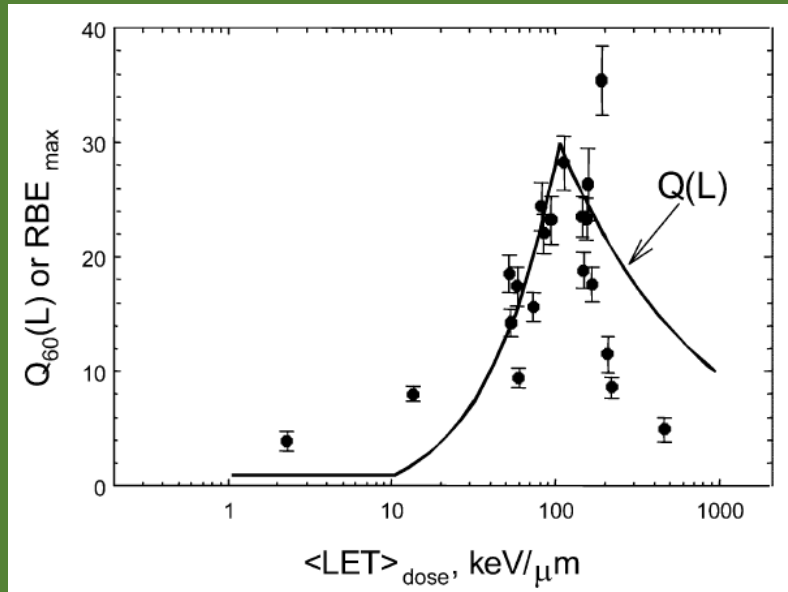


Cell: *S. Cerevisiae* (yeast)  
Exposure: beta particle. In flight  
End point: DSB induction and repair in flight  
Source: Pross et al. Rad. Res. 2000

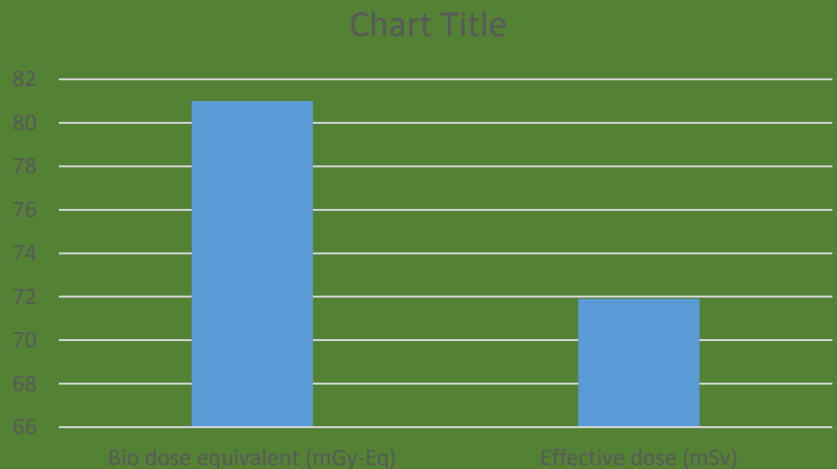


**FIG. 3.** Survival of rad54-3 yeast cells after  $\beta$ -particle exposure. Solid symbols: incubation at 22°C (permissive temperature); open symbols: incubation at 37°C (restrictive temperature). Circles: incubation under microgravity; squares: ground control. The error bars indicate standard errors for dose ( $x$  axis) derived from multiple measurements (see the Materials and Methods section) of the same source. Lines drawn are only intended to guide the eye and do not imply a functional dependence.

# Chromosome aberrations in astronauts' lymphocytes from direct exposure to space radiation



RBE for CA as a function of LET showing a similar trend as the quality factor



## Results for ISS Biological Dose Equivalent (BDE) Defined by Eq. (2) and Physical Dose Estimates

Astronaut	Biological dose equivalent, mGy-Eq		Astronaut dosimeter, mGy	Skin dose equivalent, mSv	Effective dose, mSv
	Individual based	Population based			
1	94 ± 12	128 ± 25	30.9	89.9	77.6
2	127 ± 57	84 ± 41	29.7	86.5	73.7
3	78 ± 16	81 ± 19	33.1	96.4	82.1
4	60 ± 24	87 ± 20	31.8	93.8	79.9
5	36 ± 15	54 ± 26	29.1	85.1	72.5
6	59 ± 19	61 ± 21	31.5	90.8	80.0
7	40.9 ± 19	72 ± 27	29.0	83.3	70.6
8	83 ± 29	40 ± 21	30.9	88.3	74.7
9	113 ± 17	130 ± 25	39.6	115	98.6
10	—	75 ± 26	30.7	88.3	74.5
11	74 ± 32	55 ± 26	22.2	64.5	54.7
12	128 ± 40	71 ± 24	22.5	65.4	55.7
13	134 ± 45	88 ± 29	22.3	64.7	59.8
14	66 ± 21	59 ± 15	26.3	78.0	66.3
15	83 ± 27	125 ± 52.0	29.8	88.6	75.2
16	10 ± 24	15 ± 35	20.4	56.8	47.5
17	147 ± 48	134 ± 66	36.4	103.0	86.3
18	113 ± 26	109 ± 34	29.9	83.7	76.9
19	119 ± 32	69 ± 23	23.8	70.1	59.5
Average <sup>a</sup>	85 ± 38	81 ± 32	28.9 ± 4.9	83.8 ± 14.1	71.9 ± 12.0

# **Micro-7 Project Objectives**

**Aim #1. Investigate changes of miRNA and RNA expression in G1 human fibroblast cells in space.**

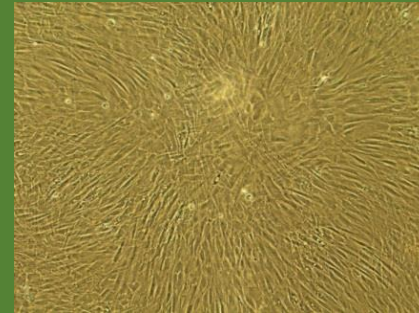
**Aim #2. Investigate cellular responses to bleomycin-induced DNA damage in G1 human fibroblast cells in space.**

**Aim #3. Detect the DNA damage in cells from direct exposure to space radiation.**

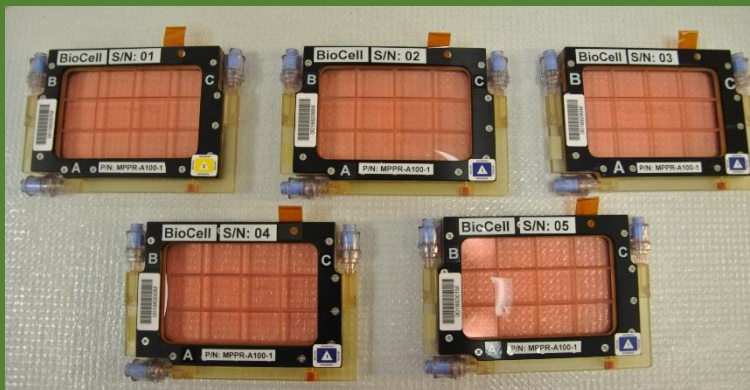
# Cell culture and flight hardware

Confluent human fibroblast cells were cultured in BioCells. The cells were kept in CGBA on ISS at 37 C.

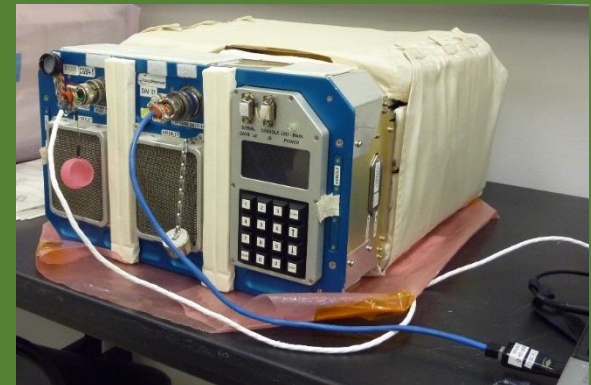
Human fibroblast cells



BioCell from BioServe



BioServe's CGBA incubator





# Flight Schedule

4/18/14 – Cells were launched to ISS on board SpaceX-3.

4/22/14 – Cells were transferred to a 37 C incubator.

4/25/14 – Cells were fixed for RNA and miRNA analysis (Day 3).

4/25/14 – Cells were treated with bleomycin (1  $\mu\text{g/ml}$ ) (Day 3).

5/6/14 – Cells were fixed for RNA and miRNA analysis (Day 14).

5/20/14 – The fixed samples returned to JSC.



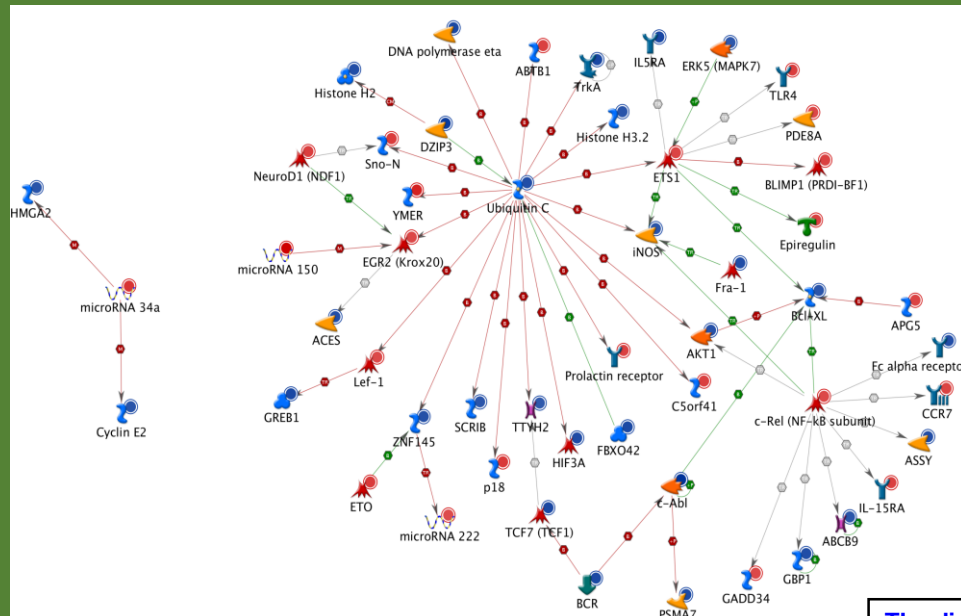
# Does spaceflight influence RNA and miRNA expression in *non-dividing* cultured cells?

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 286, NO. 37, pp. 32483–32490, September 16, 2011  
Printed in the U.S.A.

## Effects of Simulated Microgravity on Expression Profile of MicroRNA in Human Lymphoblastoid Cells<sup>\*[5]</sup>

Received for publication, June 2, 2011, and in revised form, July 18, 2011. Published, JBC Papers in Press, July 20, 2011, DOI 10.1074/jbc.M111.267765

Lingegowda S. Mangala<sup>†§1,2</sup>, Ye Zhang<sup>†¶1</sup>, Zhenhua He<sup>§</sup>, Kamal Emami<sup>†</sup>, Govindarajan T. Ramesh<sup>||</sup>, Michael Story<sup>\*\*</sup>, Larry H. Rohde<sup>§</sup>, and Honglu Wu<sup>†</sup>

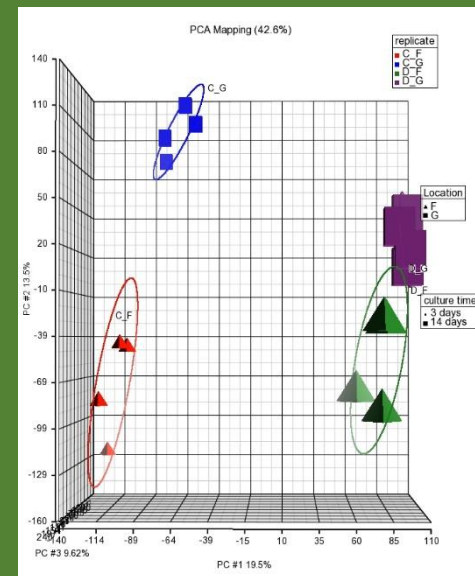
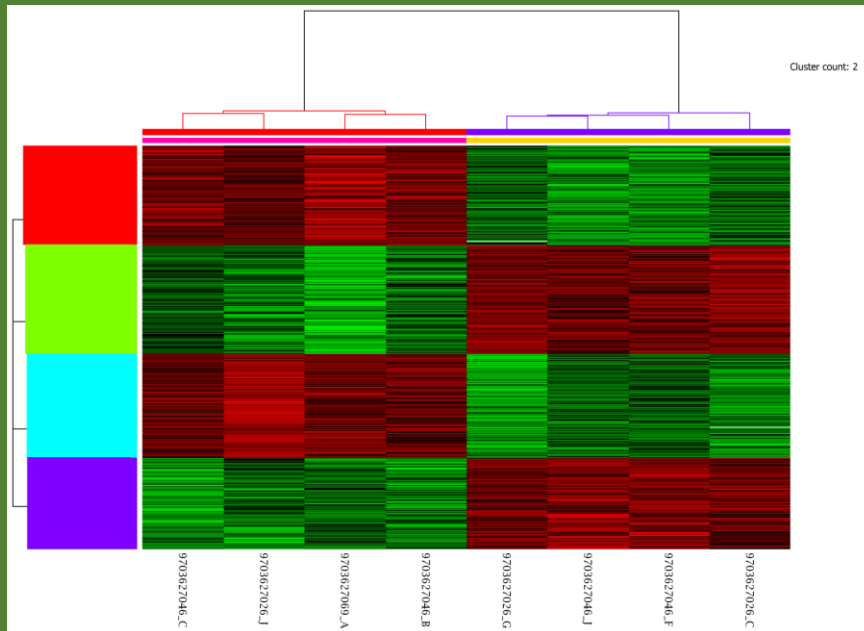
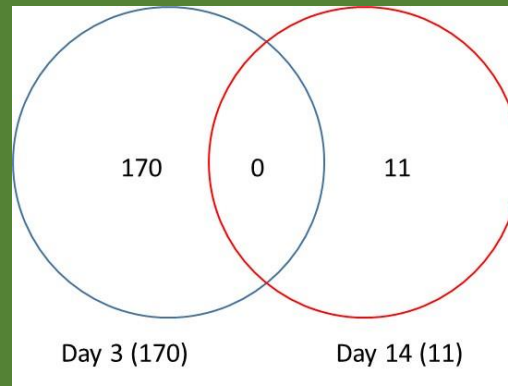


The direct interaction analysis showed several projected networks with c-Rel, ETS1 and Ubiquitin C as key factors. Several genes showed direct interactions with miRNAs that were found to be altered in simulated microgravity environment. Seven genes cyclin E2, HMGA2, EGR2, ZNF145, Ubiquitin C, ETS1 and c-Rel were subjected to validation analysis using Quantitative Real-time PCR.

**Spaceflight or simulated microgravity influences gene and miRNA expression in proliferating cells**

# Microarray Results – Day 3 and Day 14

Number of genes having significant expression changes in the flight samples in comparison to the ground controls on Day 3 and Day 14

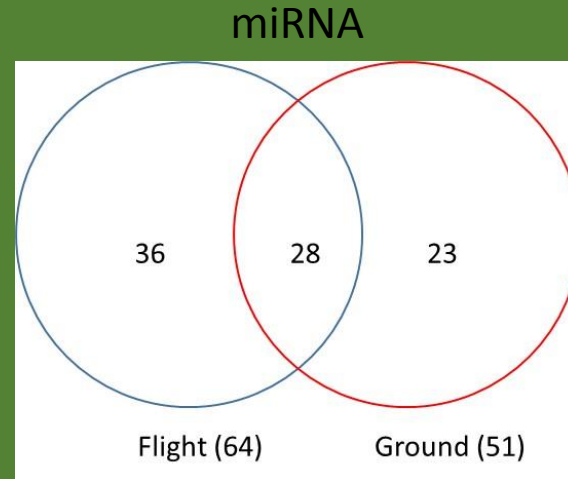
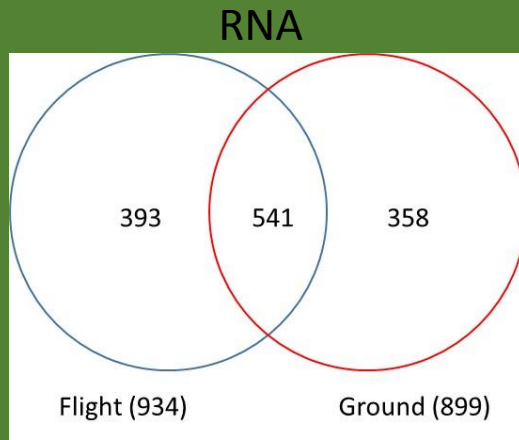


Ca<sup>2+</sup>, HGF, RELA, Vegf 1

- 
- Percent of Ki67 Positive Cells
- P < 0.01
- | Group      | Percent of Ki67 Positive Cells |
|------------|--------------------------------|
| Ground D3  | ~7.8                           |
| Ground D14 | ~4.8                           |
| Flight D3  | ~11.0                          |
| Flight D14 | ~5.0                           |

# Microarray Results – Day 3 vs. Day 14

Number of genes having significant expression changes in the Day 3 sample in comparison to the Day 14 sample.

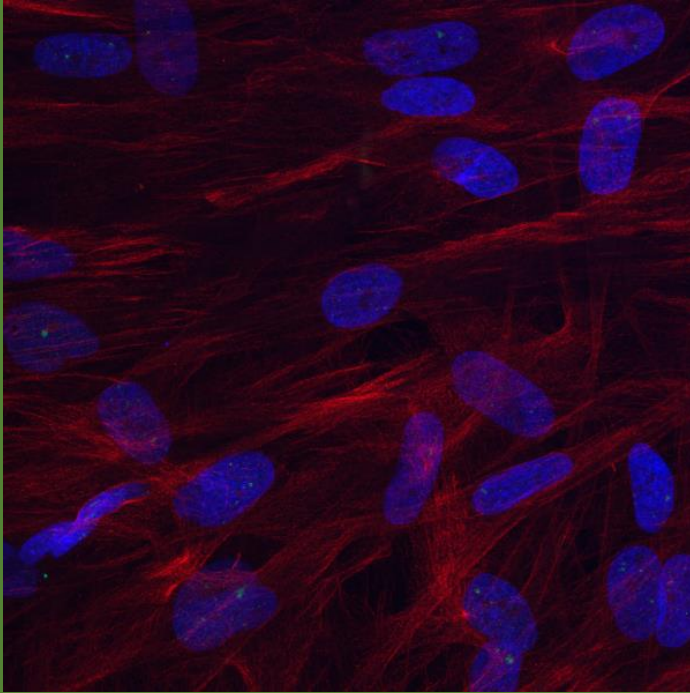


Ground		Top Upstream Regulators	Flight	
TP53	Inhibited		TP53	Inhibited
TGFb1			Vegf	Activated
dextran sulfate			HGF	Activated
CDKN1A	Inhibited		dextran sulfate	
CSF2	Activated		CDKN1A	Inhibited

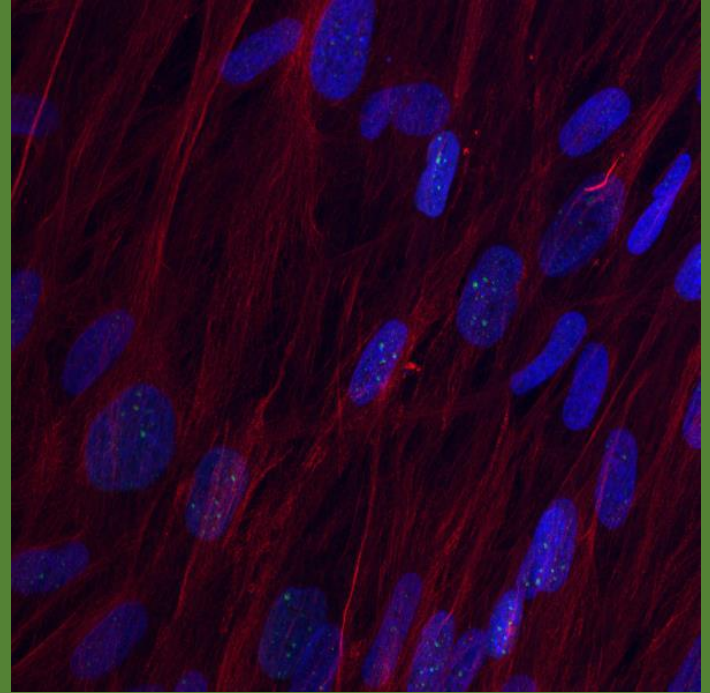
**The Day 3 cells still grew slowly even when the majority of the cells were in G1 phase.**

No significant changes in the cytoskeleton between ground and flown cells

Ground



Flight



Cells were stained with  $\alpha$ -tubulin antibodies



# Summary 1

- On Day 3, both the flown and ground cells were still proliferating slowly even though they were confluent, as measured by the expression of ki-67 positive cells, and the cells in space grew slightly faster.
- Gene and miRNA expression data for Day 3 indicated activation of NFkB and other growth related pathways involving HGF and VEGF in the flown cells.
- On Day 14 when the cells were mostly non-dividing, the gene and miRNA expression profiles between the flight and ground samples were indistinguishable.
- Comparison of gene and miRNA expressions in the Day 3 versus Day 14 samples revealed that most of the changes observed on Day 3 were related to cell growth for both the flown and ground cells.

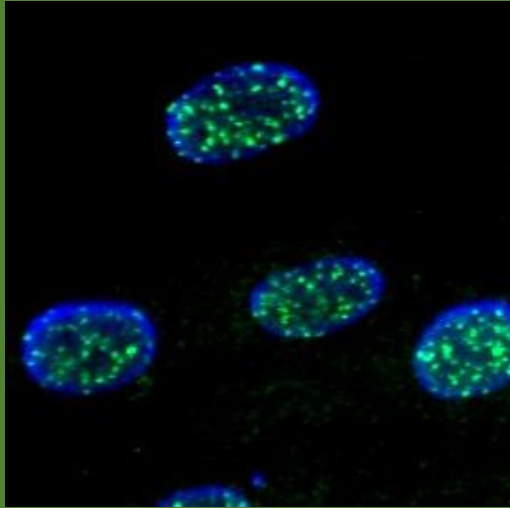
**Do microgravity and other spaceflight factors affect cellular response to DNA damages (by space radiation)?**



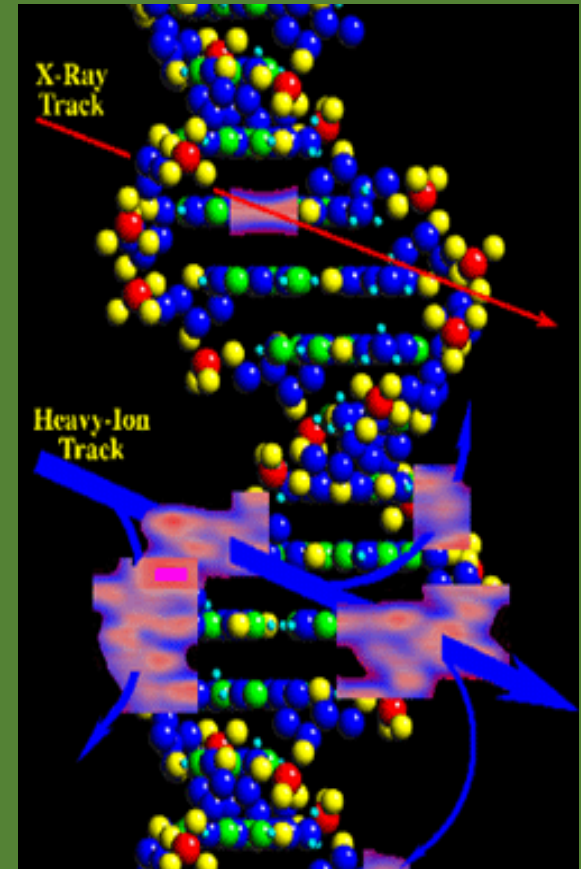
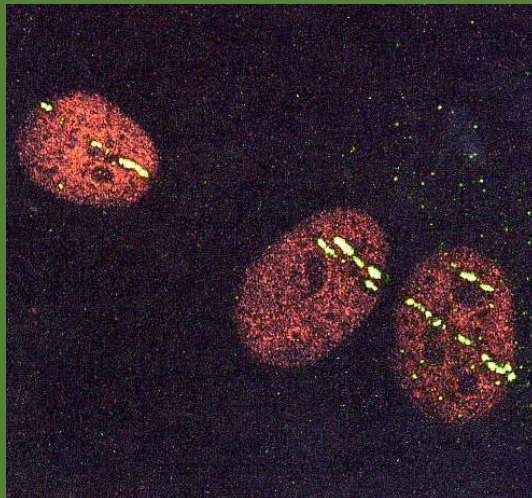
# Detection of DNA damage in the cells from direct exposure to space radiation

DSB ind

Low-LET  
X-rays  
Gamma rays

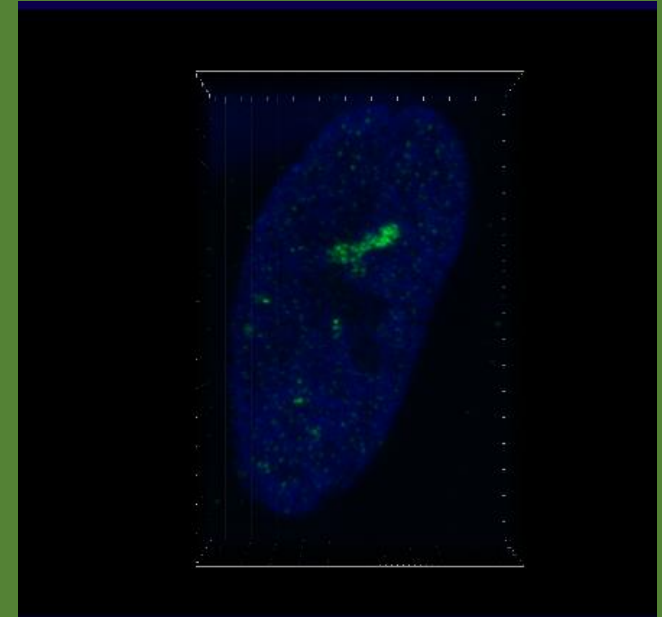
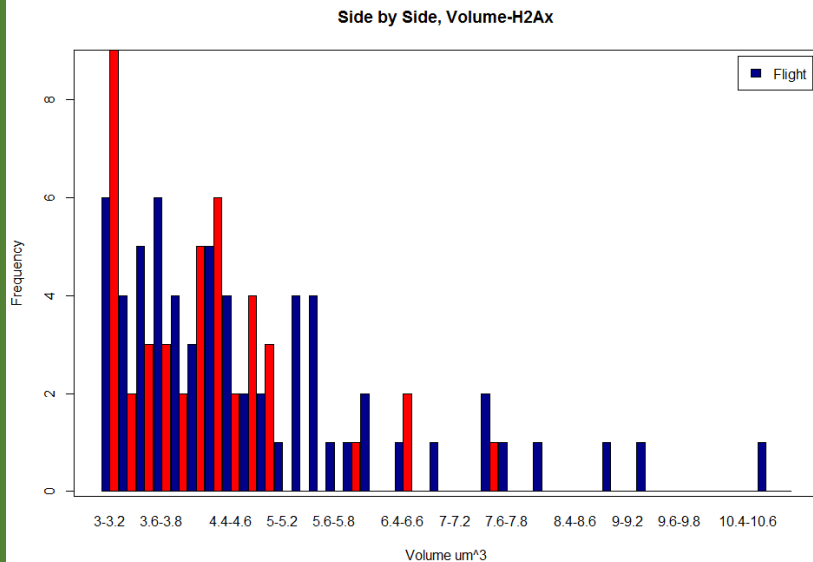
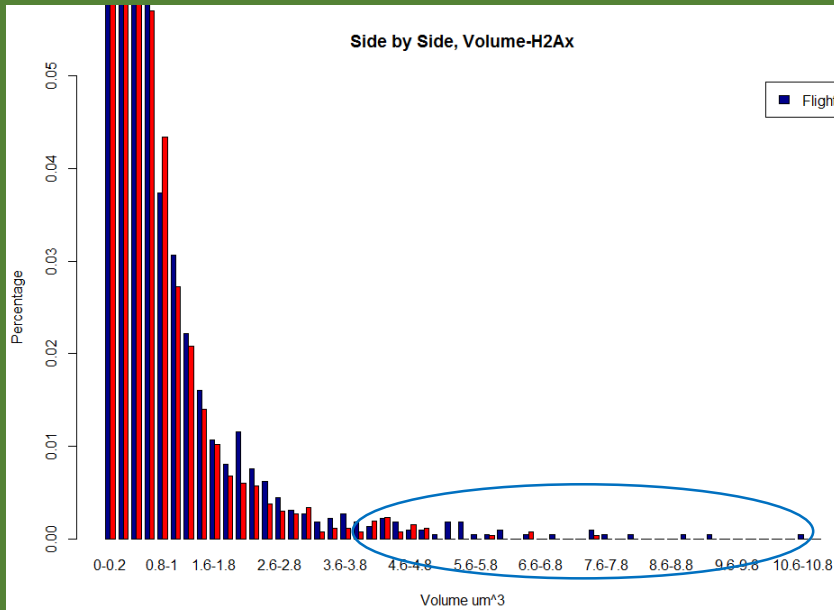


High-LET  
Space radiation



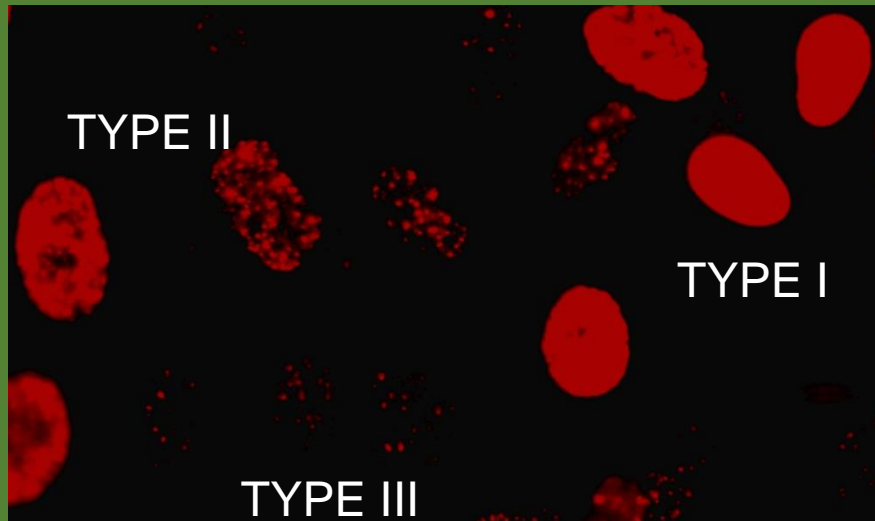
DNA damage marker –  $\gamma$ -H2AX

## Distribution of $\gamma$ -H2AX foci size



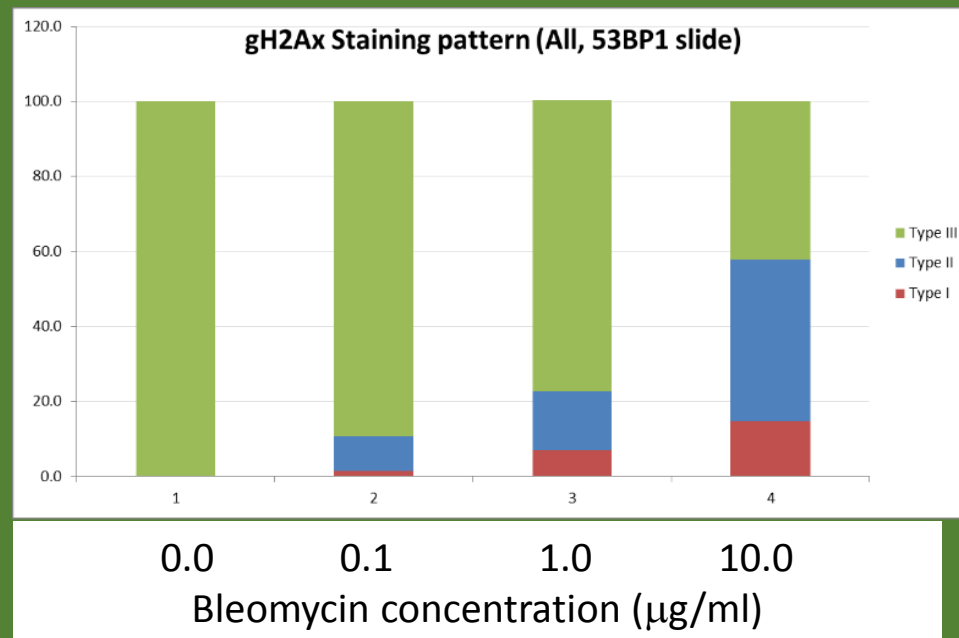
A small fraction of  $\gamma$ -H2AX foci are large and display a non-spherical track structure.

## Cellular response to bleomycin-induced DNA damage

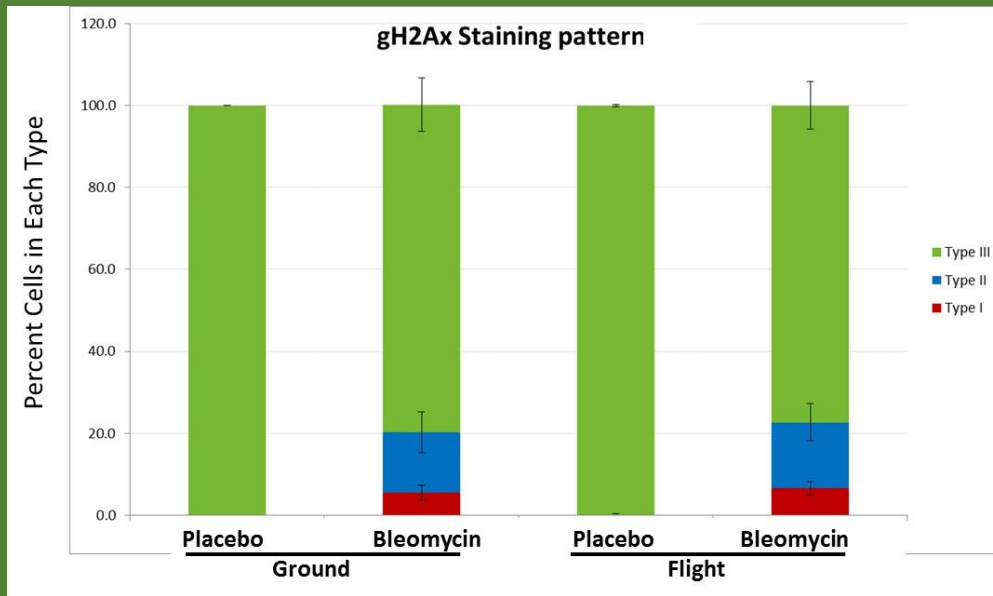


Quantification of bleomycin-induced damages with  $\gamma$ -H2AX immunofluorescence staining patterns and foci counts

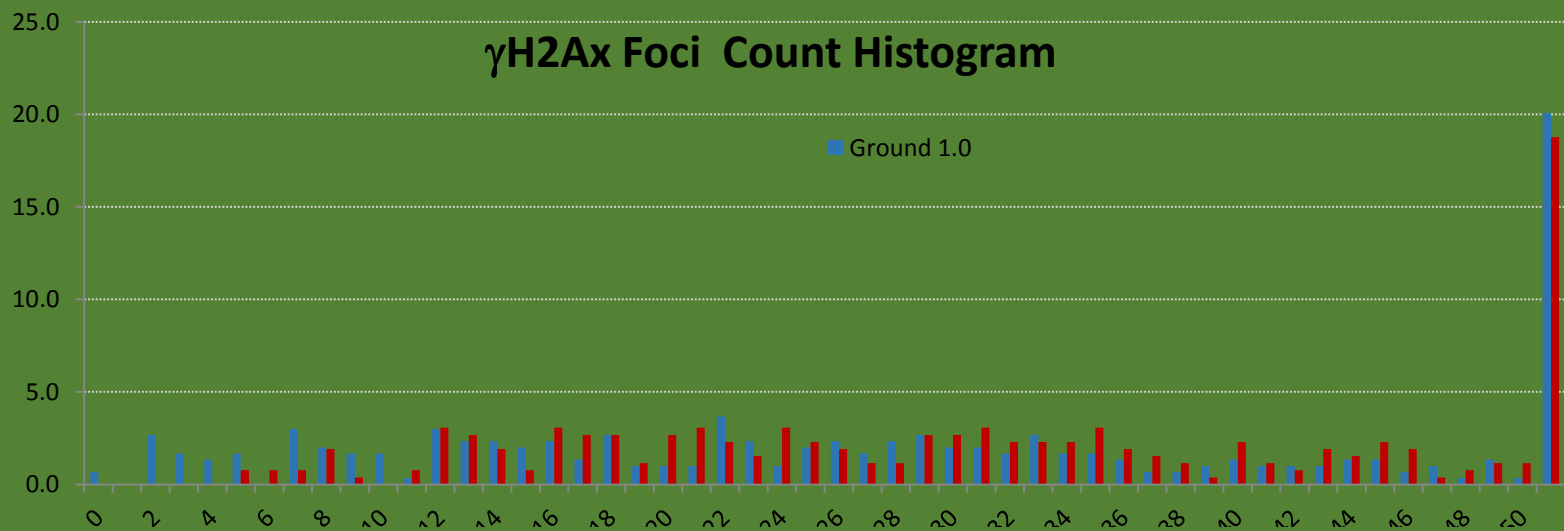
Percent Cells in Each Type



## Bleomycin results



A slight increase of the foci number per cell, as well as Type I and Type II damages, were found in the flown cells.





# Expression of genes involved in DNA damage signaling

PCRarray DNA Damage Signaling					
Ground			Flight		
BBC3	↑		BBC3	↑	
CDKN1A	↑		CDKN1A	↑	
PCNA	↑		PCNA	↑	
PPM1D	↑		PPM1D	↑	

No difference in the expression of DNA damage response genes was found between the flight and ground samples.

## Summary 2

- Images of the 3-dimensional  $\gamma$ -H2AX foci were captured with a laser confocal microscope. Quantitative analysis revealed a small fraction of foci that were larger and displayed a track pattern in the flight samples in comparison to the ground control.
- Damage in the DNA from bleomycin treatment was measured by the phosphorylation of a histone protein H2AX ( $\gamma$ -H2AX), which showed slightly more foci in the cells on ISS than in the ground control. The difference was likely caused by the slightly faster growth of the cells in space.
- Although a number of genes, including CDKN1A and PCNA, were significantly altered in the cells after bleomycin treatment, no significant differences in the expression profiles of DNA damage response genes were found between the flight and ground samples.

# Conclusions

- In true non-dividing human fibroblast cells, microgravity in space has little effect on the gene and miRNA expression. Gene and miRNA expression changes were observed in cells that were confluent, but still proliferating slowly. The faster growth in the flown cells was associated with the activation of NFkB pathways which triggers the expression of several growth factors and the suppression of the cell cycle checkpoint.
- The difference in  $\gamma$ -H2AX formation in response to bleomycin-induced DNA damage between flight and ground was due to the faster growth rate of the cells in space, but spaceflight did not affect the response of the DNA damage response genes to bleomycin treatment.

# Acknowledgement

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